

**The Biochemical Reactions of
the Tribe KLEBSIELLEAE**

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TABLE OF CONTENTS

Page No.

Acknowledgements	iii
Introduction	1
Materials and Methods	1
The Genus <i>Klebsiella</i>	2
<i>K. pneumoniae</i>	2
<i>K. ozaenae</i>	3
<i>K. rhinoschleromatis</i>	3
Differentiation of species of <i>Klebsiella</i>	3
The Genus <i>Enterobacter</i>	3
<i>E. cloacae</i>	3
<i>E. aerogenes</i>	4
<i>E. alvei</i>	4
<i>E. liquefaciens</i>	4
Differentiation of species of <i>Enterobacter</i>	4
The Genus <i>Serratia</i>	4
<i>S. marcescens</i> subspecies <i>marcescens</i>	4
<i>S. marcescens</i> subspecies <i>kiliensis</i>	5
Differentiation of <i>Klebsiella</i> , <i>Enterobacter</i> , and <i>Serratia</i>	5
Summary	5
List of Tables	
Table 1a — Sources of cultures reported	6
Table 1b — Numbers of cultures received during the 16-year period July 1, 1948 — June 30, 1964 compared to the numbers reported	6
Table 2a — Biochemical reactions given by cultures of <i>Klebsiella pneumoniae</i> (Commonly used tests)	7
Table 2b — Reactions given by strains of <i>Klebsiella pneumoniae</i> (Additional biochemical tests)	8
Table 3 — Patterns of reactions given by cultures of <i>Klebsiella pneumoniae</i> in certain differential tests and reactions obtained with strains that were somewhat aberrant	9
Table 4a — Biochemical reactions given by cultures of <i>Klebsiella ozaenae</i> (Commonly employed tests)	10
Table 4b — Reactions obtained with strains of <i>Klebsiella ozaenae</i> (Additional biochemical tests)	11
Table 5a — Patterns of reactions given by cultures of <i>Klebsiella ozaenae</i> in certain differential tests and reactions obtained with strains that were somewhat aberrant. (Based upon 51 cultures on which complete biochemical tests were made	12
Table 5b — Patterns of reactions obtained with cultures of <i>Klebsiella ozaenae</i> in certain differential tests. (Based upon 66 cultures on which incomplete data were available)	12
Table 6a — Biochemical reactions obtained with cultures of <i>Klebsiella rhinoschleromatis</i> (Commonly used tests)	13
Table 6b — Reactions given by strains of <i>Klebsiella rhinoschleromatis</i> (Additional biochemical tests)	14
Table 7a — Differentiation of species within the genus <i>Klebsiella</i> (Commonly used biochemical tests)	15

Table 7b – Differentiation of species of the genus <i>Klebsiella</i> (Additional biochemical tests)...	16
Table 8 – Differentiation within the genus <i>Klebsiella</i> (Tests of particular usefulness)	17
Table 9 – Reactions of members of the genus <i>Klebsiella</i> in sodium alginate media	17
Table 10a – Biochemical reactions of cultures of <i>Enterobacter cloacae</i> (Commonly employed tests)	18
Table 10b – Reactions obtained with strains of <i>Enterobacter cloacae</i> (Additional biochemical tests)	19
Table 11 – Patterns of reactions given by cultures of <i>Enterobacter cloacae</i> in certain differential tests and reactions obtained with strains that were somewhat aberrant	20
Table 12a – Biochemical reactions given by cultures of <i>Enterobacter aerogenes</i> (Commonly used tests)	21
Table 12b – Reactions obtained with strains of <i>Enterobacter aerogenes</i> (Additional biochemical tests)	22
Table 13 – Patterns of reactions given by cultures of <i>Enterobacter aerogenes</i> in certain differential tests and reactions obtained with strains that were somewhat aberrant	22
Table 14a – Reactions given by cultures of <i>Enterobacter alvei</i> (Commonly employed biochemical tests)	23
Table 14b – Biochemical reactions obtained with cultures of <i>Enterobacter alvei</i> (Additional tests)	24
Table 15 – Patterns of reactions given by strains of <i>Enterobacter alvei</i> in certain differential tests and reactions obtained with strains that were slightly aberrant	24
Table 16 – Differentiation of <i>Enterobacter aerogenes</i> and <i>Enterobacter alvei</i> (Biochemical tests of particular usefulness)	25
Table 17a – Biochemical tests obtained with cultures of <i>Enterobacter liquefaciens</i> (Commonly employed tests)	26
Table 17b – Reactions obtained with strains of <i>Enterobacter liquefaciens</i> (Additional biochemical tests)	27
Table 18 – Patterns of reactions obtained with cultures of <i>Enterobacter liquefaciens</i> in certain differential tests and reactions given by strains that were somewhat aberrant	28
Table 19 – Additional reactions given by cultures of <i>Enterobacter liquefaciens</i> on certain substrates	29
Table 20a – Differentiation of species of <i>Enterobacter</i> (Commonly employed biochemical tests)	30
Table 20b – Differentiation of species of <i>Enterobacter</i> (Additional biochemical tests)	31
Table 21 – Differentiation of common species of <i>Klebsiella</i> and <i>Enterobacter</i>	32
Table 22a – Biochemical reactions obtained with cultures of <i>Serratia marcescens</i> subspecies <i>marcescens</i> (Commonly used tests)	33
Table 22b – Reactions obtained with strains of <i>Serratia marcescens</i> subspecies <i>marcescens</i> (Additional biochemical tests)	34
Table 23 – Patterns of reactions given by cultures of <i>Serratia marcescens</i> subspecies <i>marcescens</i> in certain differential tests and reactions given by strains that were somewhat aberrant	35
Table 24a – Reactions given by cultures of <i>Serratia marcescens</i> subspecies <i>kiliensis</i> (Commonly used biochemical tests)	36
Table 24b – Biochemical reactions given by strains of <i>Serratia marcescens</i> subspecies <i>kiliensis</i> (Additional biochemical tests)	37
Table 25 – Differentiation of <i>Serratia marcescens</i> and <i>Enterobacter liquefaciens</i>	38
Table 26 – Differentiation of species of <i>Klebsiella</i> , <i>Enterobacter</i> and <i>Serratia</i>	39
Table 27 – Additional reactions of members of the tribe KLEBSIELLEAE on certain substrates ..	40
Table 28 – Biochemical tests of value in the differentiation of species and subspecies of KLEBSIELLEAE that give or may give negative Voges-Proskauer reactions.....	40
References	42

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The Biochemical Reactions of the Tribe KLEBSIELLEAE

INTRODUCTION

The purpose of this publication is to present the results of biochemical tests performed with relatively large numbers of cultures of each of the genera and species contained in the tribe KLEBSIELLEAE. The data obtained are presented in tabular form and the tables include the results obtained with cultures of each species in tests that are commonly employed and in additional tests of value. Further, deviations from certain patterns of reaction are listed, summaries of reactions given by the species within each genus are presented for comparative purposes, and tables listing reactions of particular value in the differentiation of species are included. The authors are hopeful that the information as well as the format in which it is presented, will prove useful to investigators in laboratories at all of the various levels. Since the tabular data are self-explanatory, comment will be limited.

The nomenclature employed is based upon that proposed by Ewing (1962, 1963) and Ewing et al. (1962) with one exception, which will be noted. This nomenclature, in turn, is based upon the taxonomic system devised by Ewing and Edwards (1960) and employed by Edwards and Ewing (1962).

No attempt was made to review the literature on the various genera of the tribe, since this was beyond the scope and purpose of the publication. However, references to recent papers are made when such references appear to be pertinent. These will serve as sources for anyone interested in delving into the literature more thoroughly.

MATERIALS AND METHODS

The cultures reported upon were among those received for identification, or for confirmation of identification, during the sixteen year period between July 1, 1948 and June 30, 1964. The sources of the 1758 strains included in this report are listed in table 1a. It should be noted (table 1a) that only a few cultures from Type Culture Collections or other collections were included, although many such strains have been studied in the past. Since only a very few cultures of *Klebsiella rhinoschleromatis* were received among diagnostic materials submitted, it was necessary to augment the authors' collection by including eleven strains which were supplied by Dr. Ida Ørskov of the State Serum Institute, Copenhagen. Although a relatively large number of cultures of *Enterobacter alvei* (Hafnia)

were received during the period mentioned, the authors nevertheless felt that it was desirable to include strains from the collections of individuals who were instrumental in the characterization of these bacteria. Hence, 20 cultures received in 1950 from the late Professor C. A. Stuart of Brown University, 4 strains from Professor Dr. F. Kauffmann and Dr. V. Møller of the State Serum Institute, Copenhagen in 1954, and 2 cultures received in 1951 (indirectly, through the late Professor R. S. Breed) from Dr. A. Castellani were included in the study. The cultures from Professor Stuart were designated type 32011 and were members of second division of *Aerobacter* (Stuart et al., 1943). The four strains received from Professor Kauffmann and Dr. Møller were representative of 30 cultures

characterized by Møller (1954). One of these four was the original strain of *B. paratyphus alvei* of Bahr (1919), which had been studied by Kauffmann and Silberstein (1934) and found not to be a *Salmonella*. Møller (1954) suggested in effect that the Bahr culture presumably ought to be regarded as the type species of *Hafnia*, under the name *Hafnia alvei*. The two cultures received indirectly from Dr. Castellani were labeled *B. asiaticus* and were identified as type 32011 by one of the writers. However, most investigators agree that the epithet *asiaticus* does not enter into the synonymy of the nomenclature of the *Hafnia* (32011) bacteria. This opinion and the reasons for it were expressed by Dr. S. T. Cowan in an Addendum (pg. 47) of the Report of the International Subcommittee on Enterobacteriaceae (1958). Since these bacteria were classified in the genus *Aerobacter* and since the generic name *Enterobacter* was conserved and *Cloacae* and *Aerobacter* were rejected*, the correct name for the *Hafnia* (32011) bacteria became *Enterobacter alvei* (Bahr) Møller (v.inf.).

In table 1b the numbers of cultures reported upon are compared to the total numbers of members of the tribe KLEBSIELLEAE received in the Enteric Bacteriology Laboratories during the sixteen year period. The 1758 cultures dealt with in this report comprized about 38 percent of the total number (4603) received (table 1b). In those instances where the number of strains received was comparatively small, all available cultures were studied (e.g., *Klebsiella ozaenae*, table 1b), whereas only a percentage (20 to 29) of the total numbers of *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *E. alvei* were included in the study.

In general, the biochemical methods employed were the recommended or standard methods given in the 1958 Report of the International Subcommittee on Enterobacteriaceae as revised and extended by Ewing (1960, 1962) and Edwards and Ewing (1962). However, several tests were employed that were not listed in the above-mentioned publications. Tests for esculin hydrolysis were made according to the method of Vaughn and Levine (1942), in which ferric citrate was incorporated into the medium. Lipolysis was studied by means of the methods employed by Hugo and Beveridge (1962) and Davis and Ewing (1964). The medium used for the detection of alginate activity was a modification of that mentioned by Skerman (1959), and

that employed in tests for utilization of alginate was prepared by substituting 0.25 percent sodium alginate for the sodium citrate in a medium similar to Simmons' citrate agar (see Davis and Ewing, 1964, for details). Tests for beta-D-galactosidase activity were made by means of the O-nitrophenyl- β -D-galactopyranoside (ONPG) procedure as employed by LeMinor and Ben Hamida (1962), Lubin and Ewing (1964), and Bülow (1964).

With the exception of the organic acid media of Kauffmann and Petersen (1956), the methods of inoculation, temperatures of incubation, and periods of incubation for the tests given in the publications of Ewing (1960, 1962) or Edwards and Ewing (1962) were employed. Multiple tubes each of sodium citrate and D-tartrate media were inoculated with each culture and tests for utilization of the substrates were made with lead acetate solution after 1, 2, 5, and 14 days of incubation according to the method of Kauffmann and Petersen (1956). Møller's medium was used for decarboxylase tests and readings were made daily for four days.

In determining the sign (+ or -) to be used in certain of the tables (e.g., 7a, 7b, 8 etc), 90 percent levels were employed, as follows:

90% or more positive results equaled +

90% or more negative results equaled -

Less than 90% positive equaled + or -, or - or +, in which instances the first sign indicated the majority.

THE GENUS KLEBSIELLA

Klebsiella pneumoniae. The results obtained in the examination of 705 cultures of *K. pneumoniae* are given in tables 2a and 2b, which are self-explanatory. Of these strains, 367 were taken from the group reported upon by Edwards and Fife (1955) and 338 were taken from materials received since that publication appeared. The results obtained with the two groups of cultures were analyzed separately and compared. No essential differences were noted, hence the results were combined.

It may be noted that 42 (6 percent) of the strains were indol positive and that 23 (3.3 percent) liquefied gelatin. Further analysis of results obtained in these two tests indicated the following relationships:

	Indol +	Indol -	Total
Gelatin +	22	1	23
Gelatin -	20	662	682
Total	42	663	705

*The Judicial Commission of the International Nomenclature Committee acted upon the request of Hormaeche and Edwards (Inter. Bull. Bact. Nomen. Tax. 10:77-78, 1960) and conserved the generic name *Enterobacter* Hormaeche and Edwards (Opinion 28, Inter. Bull. Bact. Nomen. Tax. 13:38, 1963).

The 22 cultures that liquefied gelatin and produced indol are regarded as a biotype of *K. pneumoniae* by the authors. If desired, strains of this sort could be referred to as the "oxytoca" biotype of *K. pneumoniae*. The only essential difference between the oxytoca cultures and other indol positive klebsiellae presently known is gelatin liquefaction, which is delayed. Therefore there is little doubt but that these bacteria are *Klebsiella*. However, it is believed that the oxytoca cultures are not sufficiently different to warrant status as a separate species. For additional references on this subject readers are referred to Lautrop (1956), Ørskov (1955, 1956), Hugh (1959), Cowan et al. (1960), and Ewing (1963).

The decarboxylase reactions given by the 705 cultures of *K. pneumoniae* may be summarized as follows:

No.	Lysine	Arginine	Ornithine
683	+	-	-
2	+	+	-
4	-	+	-
16	-	-	-

Thus it was apparent that 96.9 percent of the cultures studied yielded completely typical patterns of reaction in these tests.

The various patterns of reaction given by the above-mentioned 705 strains of *K. pneumoniae* with respect to gas production from inositol, adonitol, and glycerol, gelatin liquefaction, and decarboxylase tests are listed in table 3. Also listed in this table are the number of aberrant cultures in each group together with the deviations recorded. It may be noted that the first group contained 530 (75.2%) of the cultures and that these were completely typical with respect to the seven basic reactions mentioned above. Further, the strains listed in groups 2, 3, 4, 7, and 10 deviated in only one of these seven reactions. If the cultures contained in these groups are added to the 530 in the first group, the total was 659 or 93.5 percent of all the *K. pneumoniae* strains examined.

***Klebsiella ozaenae*.** The biochemical reactions obtained with 117 strains of *K. ozaenae* are given in tables 4a, 4b, 5a, and 5b. Cultures of this species are readily recognizable and may be differentiated from other *Klebsiella* species by means of the reactions listed in tables 7a, 7b, 8, and 9 and from other members of the tribe KLEBSIELLEAE that may be negative in the Voges-Proskauer tests by means of tests given in table 28.

***Klebsiella rhinoschleromatis*.** The number of cultures available to the authors for study was very limited. However, the reactions obtained in biochemical tests (tables 6a and 6b) were quite uniform and were similar to those reported by others (e.g., Ørskov, 1955) insofar as comparisons could be made.

Differentiation of species of *Klebsiella*. The biochemical reactions given by cultures of the three species of *Klebsiella* are summarized in tables 7a and 7b for comparative purposes.

Biochemical reactions of particular value in the differentiation of the three species of *Klebsiella* are listed in tables 8 and 9.

For additional data on the biochemical reactions given by cultures of *Klebsiella* and for further references, the reader is referred to Møller (1954), Edwards and Fife (1955), Ørskov (1955, 1956), Kauffmann (1954, 1956a, 1956b), Lautrop (1956), Hormaeche and Munilla (1957), Sakazaki and Namioka (1957), Hormaeche and Edwards (1958), Hugh (1959), Sedlak and Slajsova (1959), Cowan et al. (1960), Grimes (1961), and Edwards and Ewing (1962).

In passing, perhaps it should be noted that the majority of atypical or aberrant cultures of *K. pneumoniae* belong to capsular types 1 and 2 and occasionally to 3, that strains of *K. ozaenae* almost invariably belong to capsular type 4, and that cultures of *K. rhinoschleromatis* usually are members of capsular type 3. For further discussion of this matter, the reader is referred to Edwards and Ewing (1962).

THE GENUS ENTEROBACTER

***Enterobacter cloacae*.** The biochemical reactions given by the 201 cultures of *E. cloacae* studied are recorded in tables 10a and 10b. Further, the patterns of reactions observed with respect to the decarboxylases; gas production from inositol, adonitol, and glycerol; gelatin liquefaction; and motility are listed in table 11 together with the numbers of aberrant strains and deviations noted. As regards the decarboxylase reactions, 191 (95%) gave the typical pattern of reaction (- + +) for this species. It may be seen (table 11) that 96 (47.8%) of the cultures gave completely typical reactions with respect to the eight tests mentioned above. Also, it may be noted that among the 105 cultures in the other groups listed in table 11, 68 (64.8%) deviated in only one of the eight above-mentioned tests. Hence, 164 (81.6%) of the cultures gave com-

pletely typical results in these eight tests or gave atypical reactions in only one of them.

***Enterobacter aerogenes*.** Members of this species apparently occur much less commonly than *E. cloacae*. In any event, only 75 cultures of *E. aerogenes* were submitted to the laboratory during the 16 year period as compared to a much larger number of strains of *E. cloacae* (table 1b). The biochemical reactions obtained with the above-mentioned 75 cultures of *E. aerogenes* are summarized in tables 12a and 12b, and the patterns of reactions given in eight tests are listed in table 13 together with the number of aberrant strains. Attention is directed to the fact (table 13) that 73 (97.3%) of the cultures yielded decarboxylase reactions typical of the species, i.e., + - +. Further, only one culture varied from the typical pattern in more than one of the eight more important tests listed (table 13). This strain failed to decarboxylate lysine and was nonmotile. All except 17 (22.7%) of the cultures liquefied gelatin.

***Enterobacter alvei*.** Since considerable work on bacteria of this species was reported by Møller (1954) and more recently by Sakazaki (1961), the authors elected to study 100 cultures which, with the few exceptions noted above (table 1a), were taken at random from a relatively large collection (table 1b). Møller studied 30 cultures and Sakazaki included the results obtained with 294 strains in his report.

The results obtained in the investigation of the above-mentioned 100 cultures are given in tables 14a and 14b. In table 15 the strains are separated into groups according to their decarboxylase reactions; formation of gas from inositol, adonitol, and glycerol, or lack of it; gelatin liquefaction; and motility. Also, the number of strains that gave aberrant reactions in other tests is given in table 15 together with the deviations noted.

In most instances there was agreement between the results reported by Sakazaki (1961) and those listed in tables 14a and 14b. However, there were some notable differences. For example, Sakazaki included 16 (5.4%) indol positive strains and 5 (1.7%) cultures that liquefied gelatin slowly in the group of 294 which he reported upon.

Means by which cultures of *E. alvei* may be differentiated from those of *E. aerogenes* are given in table 16. The several points of difference between cultures of the two led the authors to the conclusion (Ewing, Fife, and Edwards, in press) that members of the Hafnia group should be elevated to species rank (*E. alvei*) rather than be maintained at the subspecific level as suggested

by Ewing (1963). The suggestion that these bacteria comprise a species has been made or intimated by others, e.g., Sakazaki (1961).

***Enterobacter liquefaciens*.** Sixty-eight cultures of this species were available for examination and the results of biochemical tests obtained with these are recorded in tables 17a and 17b. It should be noted that strains of *E. liquefaciens* are more reactive when incubated at 22 C than they are when incubated at 37 C. Rapid liquefaction of gelatin is a characteristic of this species of *Enterobacter*. Grimes (1961) has referred to these bacteria as cold-tolerant mesophilic strains, optimum temperature, 20 C to 30 C.

The patterns of reactions given by strains of *E. liquefaciens* in decarboxylase tests; gas production from inositol, adonitol, and glycerol; gelatin liquefaction; and motility are given in table 18. In the interest of clarity, acid and gas production by *E. liquefaciens* strains from certain substrates at incubation temperatures of 37 C and 22 C are recorded separately in table 19.

Differentiation of species of *Enterobacter*. The results obtained with the cultures of *E. cloacae*, *E. aerogenes*, *E. alvei*, and *E. liquefaciens* studied are summarized in tables 20a and 20b. In these the differences between the four species are readily discernible.

In table 21 are listed certain tests that are of particular value in the differentiation of *K. pneumoniae* and *E. cloacae*. These two were the most commonly occurring species of their respective genera among materials examined by the writers.

For additional information regarding the biochemical reactions of members of the genus *Enterobacter* and for further bibliographic sources, the reader is referred to the publications of Møller (1954), Kauffmann (1954, 1956a, 1956b), Edwards and Fife (1955), Ørskov (1955), Sakazaki and Namioka (1957), Hormaeche and Munilla (1957), Hormaeche and Edwards (1958, 1960), Sedlak and Matejovska (1958), Sedlak and Slajsova (1959), Sakazaki and Namioka (1960), Sakazaki (1961), Grimes (1961), and Edwards and Ewing (1962).

THE GENUS *SERRATIA*

Serratia marcescens subspecies *marcescens*.

The results of biochemical tests with 456 cultures of this subspecies of *S. marcescens* are summarized in tables 22a and 22b. No strains from type culture collections or other collections were included. In table 23 the patterns of reactions obtained in certain tests are listed together with the aberrant re-

actions given by some strains in other tests. It may be noted that with respect to the fermentation of arabinose and raffinose, the Voges-Proskauer test, utilization of malonate, gelatin liquefaction, and the decarboxylase tests, 406 (89%) of the cultures yielded completely typical results. Lactose fermentation was left out of consideration here because the majority of strains do not utilize this substrate, and those that do, produce weakly positive delayed reactions. Similarly, gas production was not considered since the majority of cultures are anaerogenic and because the gas volumes produced by aerogenic strains are small.

***Serratia marcescens* subspecies *kiliensis*.** Only 14 cultures of this subspecies were recognized among cultures received for identification during the period covered by this report. The biochemical reactions obtained with these strains are given in tables 24a and 24b. Attention is directed to the fact that the only important difference between *S. marcescens* subspecies *marcescens* and *S. marcescens* subspecies *kiliensis* was the failure of cultures of the latter to produce a positive Voges-Proskauer test.

Biochemical tests of particular value in the differentiation of *S. marcescens* and *E. liquefaciens* are listed in table 25.

The data presented in tables 22a, 22b, 23, 24a, 24b, 25, and 26 (v. inf.) are from the work of Ewing, Lubin, Davis, and Johnson (1965, in press).

For additional information and references concerning the genus *Serratia* the reader is referred to

Ewing, Davis, and Reavis (1959), Ewing, Davis, and Johnson (1962), Ewing, Johnson, and Davis (1962), Sedlac, Dlabac, and Motlikova (1965), Colwell and Mandel (1965), and Ewing, Lubin, Davis, and Johnson (1965, in press).

Differentiation of *Klebsiella*, *Enterobacter*, and *Serratia*. Biochemical reactions that are of particular value in the differentiation of *K. pneumoniae*, the species of *Enterobacter*, and *S. marcescens* subspecies *marcescens* are summarized in tables 26 and 27. Further, tests of value in the differentiation of species and subspecies of KLEBSIELLEAE that give or may give negative Voges-Proskauer tests are given in table 28.

SUMMARY

The biochemical reactions given by 1758 cultures of the tribe KLEBSIELLEAE (*Klebsiella*, *Enterobacter*, and *Serratia*) are summarized, primarily in tabular form, and biochemical tests of particular value in the differentiation of species within the genera are given. Twenty-eight tables are included, 13 of which are divided into parts a and b.

Table 1a.—Sources of cultures reported

Sources	<i>Klebsiella</i>			<i>Enterobacter</i>				<i>Serratia marcescens</i>
	<i>pneumoniae</i>	<i>ozaenae</i>	<i>rhinoscleromatis</i>	<i>cloacae</i>	<i>aerogenes</i>	<i>liquefaciens</i>	<i>alvei</i>	
Human:								
Pulp canal								3
Nasopharynx, nasal secretion	11	14	7	1	6			1
Sputa, oral cavity, bronchial washings, ear	161	74	1	28	29			100
Lungs, pleura, pleural fluid	42	2		2	3			4
Stools, intestinal contents	49	1		23	6	1	41	15
Gall bladder, bile	2			1				2
Urine, urinary tract	184	4		21	5	2		174
Blood	80			30	3	1		32
Spinal fluid	5			8				2
Bone, synovial fluid	1			1				7
Wounds, pus, abscesses	29			14	5		1	47
Mastitis								1
Miscellaneous (Scalp, eye, brain, pericardial fluid, etc.)	5	2		2				6
Animal and animal feeds	37			27	3	3	10	6
Miscellaneous (water, air, soil, sewage)	1			2		2	1	4
Type Culture Collections	7	3	1	1			1	
Other culture collections			11				26	
Dairy products						49		
Unknown	91	17	2	40	15	10	20	66
Total	705	117	22	201	75	68	100	470

Table 1b.—Numbers of cultures received during the 16-year period July 1, 1948 – June 30, 1964 compared to the numbers reported

Species	Number of cultures received	Cultures reported	
		Number	Percent of total received
<i>Klebsiella pneumoniae</i>	2,414	705	29
<i>K. ozaenae</i>	117	117	100
<i>K. rhinoscleromatis</i>	22	22	100
<i>Enterobacter cloacae</i>	999	201	20
<i>E. aerogenes</i>	75	75	100
<i>E. alvei</i>	379	100	26
<i>E. liquefaciens</i>	68	68	100
<i>Serratia marcescens</i>	529	470	89
Total	4,603	1,758	38

Table 2a.—*Biochemical reactions given by cultures of
Klebsiella pneumoniae (Commonly used tests)*

Test or substrate	Number of cultures					Percent				
	+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
	1-2 ¹	3-7	8-14	>14		1-2	3-7	8-14	>14	
Hydrogen sulfide	0	3	702	0	.4	99.6
Urease	666	39	94.5	5.5
Indol	42	663	6	94
Methyl red (37 C)	94	611	13.3	86.7
Voges-Proskauer (37 C)	642	63	91.1	8.9
Citrate (Simmons')	689	16	97.7	2.3
KCN	690	15	97.9	2.1
Motility	0	705	0	100
Gelatin (22 C)	23	682	3.3	96.7
Lysine decarboxylase	685	20	97.2	2.8
Arginine dihydrolase	6	699	.9	99.1
Ornithine decarboxylase	0	705	0	100
Phenylalanine deaminase	0	705	0	100
Glucose	705	0	100	0
Gas from glucose	680	25	96.5	3.5
Lactose	692	9	1	3	98.2	1.3	.14
Sucrose	697	1	7	98.91	1
Dulcitol	222	483	31.5	68.5
Salicin	703	2	0	99.7	.3	0
Adonitol:										
acid	618	87	87.7	12.3
gas	588	2	115	83.4	.3	16.3
Inositol:										
acid	690	5	1	9	97.9	.7	.1	1.3
gas	648	14	43	91.9	2	6.1
Sorbitol	701	2	2	99.4	.33
Arabinose	704	1	99.91
Raffinose	703	2	99.73
Rhamnose	700	3	2	99.3	.43

¹Days of incubation

Table 2b.—*Reactions given by strains of Klebsiella pneumoniae (Additional biochemical tests)*

Test or substrate	Number of strains					Percent				
	+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14		1-2	3-7	8-14	>14	
Malonate	652	53	92.5	7.5
Mucate	654	51	92.8	7.2
Nitrite from nitrate	704	1	99.91
Organic acids ¹ :										
citrate	454	251	64.4	35.6
D-tartrate	473	232	67.1	32.9
Jordan's tartrate (338)	319	19	94.4	5.6
Sodium alginate (90)	0	90	0	100
Lipases (90):										
Corn oil	0	90	0	100
Triacetin	1	89	1.1	98.9
Tributyrin	0	90	0	100
Pectate (90)	0	90	0	100
Starch	507	187	11	2	71.6	26.5	1.63
Xylose	701	3	1	99.5	.41
Cellobiose:										
acid	704	1	0	99.9	.1	0
gas	675	2	28	95.7	.3	4
Glycerol:										
acid	685	16	3	1	97.2	2.3	.41
gas	652	17	8	1	27	92.5	2.4	1.2	.1	3.8
Alpha methyl glucoside (95)	82	13	0	86.3	13.7	0
Erythritol (95)	0	95	0	100
Esculin (95)	94	1	0	98.9	1.1	0

¹Method of Kauffmann and Petersen, 1956

Table 3.—*Patterns of reactions given by cultures of Klebsiella pneumoniae in certain differential tests and reactions obtained with strains that were somewhat aberrant*

Number of cultures in each group	Lysine	Arginine	Ornithine	Inositol (gas)	Adonitol (gas)	Glycerol (gas)	Gelatin	Number of aberrant strains	Deviations noted
530	+	-	-	+	+	+	-	114	30 urea -; 6 citrate -; 4 citrate (+); 11 indol +; 1 urea -, sucrose -; 1 sucrose -; 1 sucrose (+); 1 citrate -, rhamnose (+); 4 rhamnose (+); 1 sorbitol -; 1 sorbitol (+); 1 cellobiose (+); 1 lactose (+), salicin (+); 25 MR +, VP -; 7MR +, VP -, indol +; 1 MR +, VP +, indol +; 1 MR +, VP -, xylose -; 1 MR +, VP -, citrate (+), lactose -; 1 MR +, VP -, citrate (+), urea -, KCN -; 1 MR +, VP -, citrate (+), sucrose -; 1 MR +, VP -, raffinose -; 6 MR +, VP -, KCN -; 1 MR +, VP -, urea -; 2 MR +, VP -, lactose -, rhamnose -; 1 MR +, VP -, arabinose -; 2 citrate -, lactose (+); 1 MR +, VP -, citrate (+).
88	+	-	-	+	-	+	-	85	75 adonitol - (acid); 3 urea -, adonitol - (acid); 2 MR +, VP + adonitol - (acid); 1 KCN -, adonitol - (acid); 2 lactose (+), adonitol - (acid); 1 citrate -, adonitol - (acid); 1 citrate (+), adonitol - (acid).
3	+	-	-	+	+	-	-	3	1 citrate (+), lactose (+), salicin (+); 1 MR +, VP -, citrate -, lactose (+); 1 lactose (+), citrate -.
14	+	-	-	-	+	+	-	10	3 MR +, VP -; 1 MR +, VP -, lactose (+); 2 xylose (+); 1 inositol - (acid); 1 MR +, VP -, KCN -; 1 indol +, MR +, VP +, sucrose (+); 1 urea -.
2	+	-	-	-	-	+	-	2	1 MR +, VP +; 1 MR +, VP -, sucrose -.
22	+	-	-	-	-	-	-	22	5 anaerogenic; 2 anaerogenic, citrate -; 1 anaerogenic, citrate (+); 3 anaerogenic, MR +, VP -; 1 anaerogenic, MR +, VP -, citrate (+); 1 anaerogenic, citrate (+), lactose (+), salicin (+); 4 anaerogenic, MR +, VP +; 1 anaerogenic MR +, VP -, adonitol - (acid); 1 MR +, VP -; 1 MR +, VP -, citrate -; 2 MR +, VP +, citrate (+).
22	+	-	-	+	+	+	+	21	19 indol +; 2 indol +, MR +, VP +
1	+	-	-	-	-	-	+	1	1 indol +, anaerogenic
4	-	+	-	+	+	+	-	4	2 MR +, VP -; 1 MR +, VP -, citrate -, sucrose -, raffinose -; 1 sucrose (+)
2	+	+	-	+	+	+	-	1	1 MR +, VP -, urea -
12	-	-	-	+	+	+	-	1	1 MR +, VP -
1	-	-	-	+	+	+	-	1	1 adonitol - (acid)
1	-	-	-	-	-	+	-	1	1 MR +, VP -, citrate -, lactose (+), sucrose (+)
2	-	-	-	-	+	+	-	2	1 MR +, VP -, sorbitol -; 1 inositol - (acid)
1	-	-	-	-	-	-	-	1	1 anaerogenic, MR +, VP -

Table 4a. — Biochemical reactions given by cultures of *Klebsiella ozaenae* (Commonly employed tests)

Test or substrate	Number tested	Number					Percent				
		+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
		1-2	3-7	8-14	<14		1-2	3-7	8-14	<14	
Hydrogen sulfide	117	0	117	0	100
Urease	117	12	13	92	10.3	11.1	78.6
Indol	117	0	117	0	100
Methyl red (37 C)	117	116	1	99.19
Voges-Proskauer (37 C)	117	0	117	0	100
Citrate (Simmons')	117	37	37	43	31.6	31.6	36.8
KCN	51	45	6	88.2	11.8
Motility	117	0	117	0	100
Gelatin (22 C)	113	0	113	0	100
Lysine decarboxylase	51	¹ 25	26	49	51
Arginine dihydrolase	51	3	48	5.9	94.1
Ornithine decarboxylase	51	1	50	2	98
Phenylalanine deaminase	51	0	51	0	100
Glucose	117	117	0	100	0
Gas from glucose	117	79	4	34	67.5	3.4	29.1
Lactose	117	28	74	8	1	6	23.9	63.3	6.8	.9	5.1
Sucrose	117	19	14	7	..	77	16.2	12	6	..	65.8
Dulcitol	117	0	117	0	100
Salicin	117	114	3	0	97.4	2.6	0
Adonitol:											
acid	117	116	1	0	99.1	.9	0
gas	51	30	1	20	58.8	2	39.2
Inositol:											
acid	117	70	24	1	..	22	59.8	20.5	.9	..	18.8
gas	51	14	4	33	27.5	7.8	64.7
Sorbitol	51	40	3	2	..	6	78.4	5.9	3.9	..	11.8
Arabinose	51	51	0	100	0
Raffinose	51	45	1	5	88.2	2	9.8
Rhamnose	51	32	4	15	62.8	7.8	29.4

¹Eight strains gave positive reactions after 3 or 4 days' incubation.

Table 4b. — *Reactions obtained with strains of Klebsiella ozaenae (Additional biochemical tests)*

Test or substrate	Number tested	Number					Percent					
		+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—	
		1-2	3-7	8-14	<14		1-2	3-7	8-14	<14		
Malonate	51	3	48	5.9	94.1	
Mucate	51	13	38	25.5	74.5	
Nitrite from nitrate	117	104	13	88.9	11.1	
Organic acids ¹ :												
citrate	51	9	42	17.6	82.4	
D-tartrate	51	20	31	39.2	60.8	
Jordan's tartrate	115	67	48	58.3	41.7	
Sodium alginate	45	0	45	0	100	
Pectate	51	0	51	0	100	
Starch	109	37	49	16	...	7	33.9	45	14.7	...	6.4	
Xylose	51	47	2	1	...	1	92.1	3.9	2	...	2	
Cellobiose:												
acid	51	50	1	98	2	
gas	51	36	15	70.6	29.4	
Glycerol:												
acid	108	74	20	3	...	11	68.5	18.5	2.8	...	10.2	
gas	51	16	12	23	31.4	23.5	45.1	
Alpha methyl glucoside	53	33	13	7	62.3	24.5	13.2	
Erythritol	53	0	53	0	100	
Esculin	53	42	3	8	79.2	5.7	15.1	

¹ Method of Kauffmann and Petersen, 1956

Table 5a. — Patterns of reactions given by cultures of *Klebsiella ozaenae* in certain differential tests and reactions obtained with strains that were somewhat aberrant (Based upon 51 cultures on which complete biochemical tests were made)

Number of Cultures	Lysine	Arginine	Ornithine	Inositol (gas)	Adonitol (gas)	Glycerol (gas)	Gelatin	Motility	Number of Aberrant strains	Deviations noted
9	+	-	-	+	+	+	-	-	1	1 KCN -
4	+	-	-	-	+	+	-	-	3	3 rhamnose -
10	+	-	-	-	+	+	-	-	10	1 sorbitol -, 1 rhamnose -, 8 anaerogenic
1	+	-	-	-	-	+	-	-	1	1 nitrate -
2	-	-	-	+	+	+	-	-	2	1 KCN -, 1 rhamnose -
8	-	-	-	-	+	+	-	-	8	1 rhamnose -, sorbitol -; 2 KCN -, rhamnose -; 3 rhamnose -; 2 nitrate -, rhamnose -
1	-	-	-	+	-	+	-	-	1	1 cellobiose -
4	-	-	-	+	+	-	-	-	1	1 sorbitol -
1	-	-	-	-	+	-	-	-	1	1 KCN -, xylose -, raffinose -, rhamnose -, sorbitol -
7	-	-	-	-	-	-	-	-	6	1 anaerogenic; 3 raffinose -, anaerogenic; 1 raffinose -, rhamnose -, sorbitol -, anaerogenic; 1 sorbitol -, anaerogenic
2	-	+	+	+	+	+	-	-	1	1 nitrate -
1	+	-	+	-	-	-	-	-	1	1 KCN -, anaerogenic

Table 5b. — Patterns of reactions obtained with cultures of *Klebsiella ozaenae* in certain differential tests (Based upon 66 cultures on which incomplete data were available)

Number of Cultures	Inositol (gas)	Adonitol (gas)	Glycerol (gas)	Gelatin	Number of aberrant strains	Deviations noted
26	+	+	+	-	1	1 nitrate -
1	+	+	+	NT ¹
7	+	+	NT ¹	-	1	1 nitrate -
4	+	+	-	-
3	-	-	+	-	2	2 nitrate -
1	-	+	NT ¹	-
7	-	+	+	-	2	2 nitrate -
1	-	+	-	-
12	-	-	-	-	12	11 anaerogenic; 1 anaerogenic, nitrate -, MR -, VP-
1	-	-	NT ¹	-	1	1 anaerogenic
3	-	-	-	NT ¹	3	2 anaerogenic; 1 anaerogenic, nitrate -

¹NT=no test.

Table 6a. — *Biochemical reactions obtained with cultures of Klebsiella rhinoschleromatis*
(Commonly used tests)

Test or substrate	Number				
	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	> 14	
Hydrogen sulfide	0	22
Urease	0	22
Indol	0	22
Methyl red (37 C)	22	0
Voges-Proskauer (37 C)	0	22
Citrate (Simmons')	0	22
KCN	8	14	0
Motility	0	22
Gelatin (22 C)	0	22
Lysine decarboxylase	0	22
Arginine dihydrolase	0	22
Ornithine decarboxylase	0	22
Phenylalanine deaminase	0	22
Glucose	22	0
Gas from glucose	0	22
Lactose	0	8	8	6
Sucrose	15	7	0
Mannitol	22	0
Dulcitol	0	22
Salicin	22	0
Adonitol	22	0
Inositol	21	1	0
Sorbitol	22	0
Arabinose	22	0
Raffinose	15	7	0
Rhamnose	21	1	0

Table 6b.—*Reactions given by strains of Klebsiella rhinoschleromatis*
(Additional biochemical tests)

Test or substrate	Number				
	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14	
Malonate	21	1
Mucate	0	22
Nitrite from nitrate	22	0
Organic acids ¹ :					
citrate	0	22
D-tartrate	0	22
Jordan's tartrate (11)	4	7
Sodium alginate	0	22
Lipases (13):					
corn oil.	0	13
Triacetin	0	13
Tributyrin	0	13
Pectate	0	13
Starch	22 ^w	0
Xylose	22	0
Cellobiose	22	0
Glycerol	22	0
Alpha methyl glucoside	0	22
Erythritol	0	22
Esculin	21	1	0
Beta galactosidase	22	0

¹Method of Kauffmann and Petersen, 1956.

w = weakly positive reactions.

Table 7a.—*Differentiation of species within the genus Klebsiella*
(Commonly used biochemical tests)

Test or substrate	<i>K. pneumoniae</i>		<i>K. ozaenae</i>		<i>K. rhinoschleromatis</i>	
	Index ¹	%+ (%+) ²	Index ¹	%+ (%+) ²	Index ¹	%+ (%+) ²
Hydrogen sulfide.....	-	(4)	-	0	-	0
Urease	+	94.5	d	9.5(10.3)	-	0
Indol	-	94	-	0	-	0
Methyl red 37 C	- or +	13.3	+	99.1	+	100
Voges-Proskauer 37C	+	91.1	-	0	-	0
Citrate (Simmons')	+	97.7	d	31.9(31)	-	0
KCN (growth)	+	97.9	+ or -	88	+	100
Motility	-	0	-	0	-	0
Gelatin 22 C	-	3.3	-	0	-	0
Lysine decarboxylase	+	97.2(2.8)	- or +	48	-	0
Arginine dihydrolase	-	.9	-	6	-	0
Ornithine decarboxylase	-	0	-	4	-	0
Phenylalanine deaminase	-	0	-	0	-	0
Glucose:						
acid	+	100	+	100	+	100
gas	+	96.5	d	64 (2)	-	0
Lactose	+	98.2(1.4)	d	24.1(70.7)	(+) or -	(72.8)
Sucrose	+	98.9	d	16.3(17.3)	+ or (+)	68.2(31.8)
Mannitol	+	100	+	100	+	100
Dulcitol	- or +	31.5	-	0	-	0
Salicin	+	99.7(.3)	+	97.4(2.6)	+	100
Adonitol:						
acid	+ or -	87.7	+	98.3(1.7)	+	100
gas	d	83.4(.3)	d	60 (2)	-	0
Inositol:						
acid	+	97.9(.8)	d	58.6(21.6)	+	95.5(4.5)
gas	+	91.9(2)	d	28 (8)	-	0
Sorbitol	+	99.4(.3)	d	78 (10)	+	100
Arabinose	+	99.9	+	100	+	100
Raffinose	+	99.7	+	90	+ or (+)	68.2(31.8)
Rhamnose	+	99.3(.4)	d	60 (8)	+	95.5(4.5)

¹Index:

- +, positive within 1 or 2 days' incubation
- (+), positive reaction after 3 or more days
- , no reaction
- + or -, majority of strains positive, occasional cultures negative
- or +, majority of cultures negative, occasional strains positive
- (+) or +, majority of reactions delayed, some occur within 1 or 2 days
- d, different reactions: +, (+), -

²Numerals in parentheses indicate percentage of delayed reactions (3 or more days)

Table 7b.—*Differentiation of species of the genus Klebsiella*
(Additional biochemical tests)

Test or substrate	<i>K. pneumoniae</i>		<i>K. ozaenae</i>		<i>K. rhino- schleromatis</i>	
	Index ¹	%+ (%) ²	Index ¹	%+ (%) ²	Index ¹	%+ (%) ²
Malonate	+	92.5	—	4	+	95.5
Mucate	+	92.8	— or +	24	—	0
Nitrite from nitrate	+	99.9	+	92	+	100
Organic acids ³ :						
Citrate	+ or —	64.4	— or +	18	—	0
D-tartrate	+ or —	67.1	— or +	36	—	0
Jordan's tartrate	+	94.4	+ or —	56.9	— or +	36.4
Sodium alginate, synthetic	+ or —	88.5(9.2)	— or (+)	(37.5)	—	0
nutrient	—	0	—	0	—	0
Lipases:						
corn oil	—	0	—	0	—	0
Triacetin	—	1.1	—	0	—	0
Tributyrin	—	0	—	0	—	0
Pectate	—	0	—	0	—	0
Starch	+ or (+)	71.6(28.1)	d	34.3(59.3)	+ ^w	100
Xylose	+	99.5(.4)	+	92 (6)	+	100
Cellobiose:						
acid	+	99.9(.1)	+	98	+	100
gas	+	95.7(.3)	+ or —	70	—	0
Glycerol:						
acid	+	97.2(2.7)	d	68.2(21.5)	+	100
gas	+	92.5(3.7)	d	30 (24)	—	0
Alpha methyl glucoside	+ or (+)	86.3(13.7)	+ or (+)	75 (25)	—	0
Erythritol	—	0	—	0	—	0
Esculin	+	98.9(1.1)	+ or —	75	— or +	15.4

¹Index:

- +, positive within 1 or 2 days' incubation
- (+), positive reaction after 3 or more days
- , no reaction
- + or —, majority of strains positive, occasional cultures negative
- or +, majority of cultures negative, occasional strains positive
- (+) or +, majority of reactions delayed, some occur within 1 or 2 days
- d, different reactions: +, (+), —

²Numerals in parentheses indicate percentage of delayed reactions (3 or more days)

³Method of Kauffmann and Petersen, 1956

Table 8.—*Differentiation within the genus Klebsiella (Tests of particular usefulness)*

Test or substrate	<i>K. pneumoniae</i>		<i>K. ozaenae</i>		<i>K. rhinoschleromatis</i>	
	Index ¹	%+ (%+) ²	Index ¹	%+ (%+) ²	Index ¹	%+ (%+) ²
Urease	+	94.5	d	9.5 (10.3)	-	0
Methyl red	- or +	13.3	+	99.1	+	100
Voges-Proskauer	+	91.1	-	0	-	0
Citrate (Simmons')	+	97.7	d	31.9 (31)	-	0
Organic acids ³ :						
citrate	+ or -	64.4	- or +	18	-	0
D-tartrate	+ or -	67.1	- or +	36	-	0
Malonate	+	92.5	-	4	+	95.5
Mucate	+	92.8	- or +	24	-	0
Lysine decarboxylase	+	97.2	- or +	48	-	0
Gas from glucose	+	96.5	d	64 (2)	-	0
Lactose	+	98.2 (1.4)	d	24.1 (70.7)	(+) or -	(72.8)
Dulcitol	- or +	31.5	-	0	-	0

¹Index:

- +, positive within 1 or 2 days' incubation
 (+), positive reaction after 3 or more days
 -, no reaction
 + or -, majority of strains positive, occasional cultures negative
 - or +, majority of cultures negative, occasional strains positive
 (+) or +, majority of reactions delayed, some occur within 1 or 2 days
 d, different reactions: +, (+), -

²Numerals in parentheses indicate percentage of delayed reactions (3 or more days)³Method of Kauffmann and Petersen (1956)Table 9. — *Reactions of members of the genus Klebsiella in sodium alginate media*

Species	Number tested	Nutrient medium			Synthetic medium		
		+	(+) 3-7	-	+	(+) 3-7	-
<i>K. pneumoniae</i>	90	0	0	90	80(88.9)	8(8.9)	2(2.2)
<i>K. ozaenae</i>	45	0	0	45	0	¹ 5(11)	40(89)
<i>K. rhinoschleromatis</i>	22	0	0	22	0	0	22

¹ These 5 strains gave doubtful or weakly positive reactions after 3 to 7 days.

N.B., Numerals in parentheses are percentages of the numbers tested

Table 10a. — Biochemical reactions of cultures of *Enterobacter cloacae* (Commonly employed tests)

Test or substrate	Number					Percent				
	+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14		1-2	3-7	8-14	>14	
Hydrogen sulfide	0	201	0	100
Urease	130	71	64.7	35.3
Indol	1	200	.5	99.5
Methyl red (37 C)	6 ^w	95	.3	97
Voges-Proskauer (37 C)	200	1	99.55
Citrate (Simmons')	200	1	99.55
KCN	197	4	98	2
Motility	190	11	94.5	5.5
Gelatin (22 C)	2	40	114	39	6	1	19.9	56.7	19.4	3
Lysine decarboxylase	1	200	.5	99.5
Arginine dihydrolase	193	8	96.5	3.5
Ornithine decarboxylase	194	7	96	4
Phenylalanine deaminase	0	201	0	100
Glucose	201	0	100	0
Gas from glucose	201	0	100	0
Lactose	188	11	2	93.5	5.5	1
Sucrose	194	1	6	96.5	.5	3
Dulcitol	26	175	12.9	87.1
Salicin	152	37	12	75.6	18.4	6
Adonitol (acid and gas)	57	144	28.4	71.6
Inositol:										
acid	44	25	132	21.9	12.4	65.7
gas	9	192	4.5	95.5
Sorbitol	190	...	1	...	10	94.55	...	5
Arabinose	200	1	99.55
Raffinose	195	6	97	3
Rhamnose	185	3	13	92	1.5	6.5

^w= weakly positive reactions

Table 10b. — *Reactions obtained with strains of Enterobacter cloacae (Additional biochemical tests)*

Test or substrate	Number					Percent				
	+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14		1-2	3-7	8-14	>14	
Malonate	162	39	80.6	19.4
Mucate	152	49	75.6	24.4
Nitrite from nitrate	201	0	100	0
Organic acids ¹ :										
citrate	173	28	86.1	13.9
D-tartrate	34	167	16.9	83.1
Jordan's tartrate (198)	55	143	27.8	72.2
Sodium alginate (133)	0	133	0	100
Lipases: (133)										
corn oil	1 ^w	2	130	.8	1.5	97.7
Triacetin	0	133	0	100
Tributyrin	0	1	132	0	.8	99.2
Pectate (133)	0	133	0	100
Starch	32	34	28	5	102	15.9	17	13.9	2.5	50.7
Xylose	97	1	3	98	.5	1.5
Cellobiose:										
acid	201	0	100	0
gas	201	0	100	0
Glycerol:										
acid	87	75	15	...	24	43.3	37.3	7.5	11.9
gas	11	10	22	...	158	5.5	5	10.9	78.6
Alpha methyl glucoside (133)	112	17	4	84.2	12.8	3
Erythritol (133)	0	133	0	100
Esculin (133)	39	94	29.3	70.7

¹ Method of Kauffmann and Petersen, 1956^w=weakly positive reactions

Table 11.—*Patterns of reactions given by cultures of Enterobacter cloacae in certain differential tests and reactions obtained with strains that were somewhat aberrant*

Number of cultures in each group	Lysine	Arginine	Ornithine	Inositol (gas)	Adonitol (gas)	Glycerol (gas)	Gelatin	Motility	Number of aberrant strains	Deviations noted
96	-	+	+	-	-	-	(+)	+	50	1 MR +, VP -; 1 arabinose -; 3 xylose -; 3 lactose (+); 1 lactose -, sucrose -; 2 lactose (+), salicin (+); 1 lactose (+), salicin -; 1 sucrose (+); 2 sucrose -, raffinose -; 1 sucrose -, raffinose -, sorbitol -; 10 rhamnose -; 6 salicin -; 11 salicin (+); 1 salicin (+), raffinose -; 1 salicin (+), raffinose -, sorbitol -; 4 KCN -; 1 indol +
33	-	+	+	-	+	-	(+)	+	12	3 lactose (+), salicin (+); 8 salicin (+); 1 salicin -
5	-	+	+	+	-	-	(+)	+	4	4 sorbitol -
22	-	+	+	-	-	+	(+)	+	10	3 lactose (+), salicin (+); 1 lactose -, sucrose -; 1 sucrose -, raffinose -, rhamnose -; 1 rhamnose -; 1 rhamnose (+); 1 rhamnose (+), salicin (+); 1 salicin (+)
1	-	+	+	+	+	+	(+)	+
1	-	+	+	+	-	+	(+)	+
16	-	+	+	-	+	+	(+)	+	7	3 salicin (+); 3 salicin -, 1 citrate -
2	-	+	+	-	-	-	-	+	2	1 lactose (+); 1 sorbitol -
3	-	+	+	-	-	+	-	+	1	1 salicin (+)
1	-	+	+	-	+	-	-	+
6	-	+	+	-	-	-	(+)	-	3	1 xylose (+), 1 salicin -, 1 salicin (+)
2	-	+	+	+	-	-	(+)	-	2	2 sorbitol -
1	-	+	+	-	+	-	(+)	-	1	1 salicin (+)
2	-	+	+	-	-	+	(+)	-	1	1 rhamnose -
1	-	-	+	-	-	-	(+)	+
1	-	-	+	-	+	-	(+)	+
1	-	+	-	-	+	-	(+)	+	1	1 sorbitol (+), salicin (+)
1	+	+	+	-	+	+	(+)	+	1	1 salicin (+)
3	-	-	-	-	-	-	(+)	+
1	-	-	-	-	-	+	(+)	+
2	-	-	-	-	+	-	(+)	+

Table 12a. — Biochemical reactions given by cultures of *Enterobacter aerogenes* (Commonly used tests)

Test or substrate	Number					Percent				
	+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14		1-2	3-7	8-14	>14	
Hydrogen sulfide	0	2 ^w	73	0	2.7	97.3
Urease	2	73	2.7	97.3
Indol	0	75	0	100
Methyl red (37 C)	0	75	0	100
Voges-Proskauer (37 C)	75	0	100	0
Citrate (Simmons')	73	2	93.7	2.7
KCN	74	1	98.7	1.3
Motility	73	2	97.3	2.7
Gelatin (22 C)	0	...	15	43	17	0	...	20	57.3	22.7
Lysine decarboxylase	74	1	98.7	1.3
Arginine dihydrolase	0	75	0	100
Ornithine decarboxylase	74	1	98.7	1.3
Phenylalanine deaminase	0	75	0	100
Glucose	75	0	100	0
Gas from glucose	75	0	100	0
Lactose	69	4	2	92.1	5.3	2.6
Sucrose	75	0	100	0
Dulcitol	3	72	4	96
Salicin	74	1	0	98.7	1.3	0
Adonitol (acid and gas)	74	1	98.7	1.3
Inositol (acid and gas)	75	0	100	0
Sorbitol	75	0	100	0
Arabinose	75	0	100	0
Raffinose	72	3	96	4
Rhamnose	74	1	98.7	1.3

^w=weakly positive reaction

Table 12b. — Reactions obtained with strains of *Enterobacter aerogenes* (Additional biochemical tests)

Test or substrate	Number					Percent				
	+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14		1-2	3-7	8-14	>14	
Malonate	56	19	74.7	25.3
Mucate	71	4	94.7	5.3
Nitrite from nitrate	75	0	100	0
Organic acids ¹ :										
citrate	73	2	97.3	2.7
D- tartrate	30	45	40	60
Jordan's tartrate	67	8	89.3	10.7
Sodium alginate (49)	0	49	0	100
Lipases (49):										
corn oil	0	49	0	100
Triacetin	1	48	2	98
Tributyrin	0	49	0	100
Pectate (49)	0	49	0	100
Starch	29	6	4	5	31	38.7	8	5.3	6.7	41.3
Xylose	75	0	100	0
Cellobiose (acid and gas)	74	1	98.7	1.3
Glycerol:										
acid	75	0	100	0
gas	74	1	0	98.7	1.3	0
Alpha methyl glucoside (49)	47	1	1	96	2	2
Erythritol (49)	0	49	0	100
Esculin (49)	48	1	98	2

¹ Method of Kauffmann and Petersen, 1956Table 13.—Patterns of reactions given by cultures of *Enterobacter aerogenes* in certain differential tests and reactions obtained with strains that were somewhat aberrant

No. of Cultures	Decarboxylases			Gas from:			Gelatin	Motility	No. aberrant strains	Deviations noted
	Lysine	Arginine	Ornithine	Inositol	Adonitol	Glycerol				
54	+	—	+	+	+	+	(+)	+	12	1 KCN —, 3 raffinose —, 1 gas from raffinose (+), 2 lactose (+), 4 gas from lactose (+), 1 citrate —
1	+	—	+	+	—	+	(+)	+	1	1 salicin (+)
17	+	—	+	+	+	+	—	+	2	1 gas from lactose (+); 1 cellobiose (+), rhamnose —, gas from raffinose (+)
1	+	—	—	+	+	+	(+)	+
1	—	—	+	+	+	+	(+)	—
1	+	—	+	+	+	+	(+)	—

Table 14a. — *Reactions given by cultures of Enterobacter alvei (Commonly employed biochemical tests)*

Test or substrate	Number				
	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14	
Hydrogen sulfide	0	100
Urease	3 ^w	97
Indol	0	100
Methyl red:					
37 C	54	46
22 C	1	99
Voges-Proskauer:					
37 C	65	35
22 C	99	1
Citrate (Simmons*):					
37 C	0	50	7	1	42
22 C	3	72	7	0	18
KCN	97	3
Motility	93	7
Gelatin (22 C)	0	100
Lysine decarboxylase:					
37 C	100	0
22 C	100	0
Arginine dihydrolase:					
37 C	9	91
22 C	5	95
Ornithine decarboxylase:					
37 C	100	0
22 C	100	0
Phenylalanine deaminase	0	100
Glucose	100	0
Gas from glucose	100	0
Lactose	0	5	14	4	77
Sucrose	12	31	22	8	27
Mannitol	100	0
Dulcitol	1	99
Salicin	13	2	5	1	79
Adonitol	0	100
Inositol	0	100
Sorbitol	0	100
Arabinose	96	4
Raffinose	0	100
Rhamnose	93	7	0

^w=weakly positive reaction

Table 14b. — Biochemical reactions obtained with cultures of *Enterobacter alvei* (Additional tests)

Test or substrate	Number				
	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14	
Malonate	74	26
Mucate	0	100
Nitrite from nitrate	100	0
Organic acids ¹ :					
citrate	99	1
D-tartrate	0	100
Jordan's tartrate (57)	43	14
Sodium alginate (125)	0	125
Lipases (125)					
corn oil	0	125
Triacetin	0	125
Tributyrin	0	125
Pectate (121)	0	121
Starch	50 ^w	14 ^w	1 ^w	1	34
Xylose	97	3
Cellobiose:					
acid	76 ^w	8	7	1	8
gas	67 ^w	14	6	2	11
Glycerol:					
acid	100	0
gas	95	5	0
Alpha methyl glucoside	0	100
Erythritol	0	100
Esculin	6	2	92
Beta galactosidase (39 ²)	39	0

¹ Method of Kauffmann and Petersen, 1956² Only strains that failed to ferment lactose were tested^w=weakly positive reactionTable 15.—Patterns of reactions given by strains of *Enterobacter alvei* in certain differential tests and reactions obtained with strains that were slightly aberrant

No. of Cultures	Decarboxylases			Gas from:			Gelatin	Motility	No. aberrant strains	Deviations noted
	Lysine	Arginine	Ornithine	Inositol	Adonitol	Glycerol				
84	+	—	+	—	—	+	—	+	12	3 KCN —, 5 cellobiose —, 1 Arabinose —, 2 xylose —
6	+	—	+	—	—	+	—	—	2	1 arabinose —, cellobiose —; 1 xylose —
9	+	(+ ^w)	+	—	—	+	—	+	1	1 cellobiose —
1	+	—	+	—	—	+	—	+	1	dulcitol + ₂

^w = weakly positive reaction

Table 16.—*Differentiation of Enterobacter aerogenes and Enterobacter alvei*
(Biochemical tests of particular usefulness)

Substrate or test	<i>E. aerogenes</i>		<i>E. alvei</i>	
	Index ¹	%+ (%+) ²	Index ¹	%+ (%+) ²
Adonitol:				
acid	+	98.7	—	0
gas	+	98.7	—	0
Inositol:				
acid	+	100	—	0
gas	+	100	—	0
Sorbitol	+	100	—	0
Raffinose	+	96	—	0
Salicin	+	98.7(1.3)	d	13(8)
Alpha methyl glucoside	+	96(2)	—	0
Esculin	+	98	—	6(2)
Methyl red:				
37 C	—	0	+ or —	54
22 C	—	1
Voges-Proskauer:				
37 C	+	100	+ or —	65
22 C	+	99
Citrate (Simmons ³):				
37 C	+	93.7	(+) or —	(58)
22 C	d	3(79)
Gelatin: 22 C	(+) or —	(77.3)	—	0
Mucate	+	94.7	—	0

¹Index:

- + , positive within 1 or 2 days' incubation
- (+) , positive reaction after 3 or more days
- , no reaction
- + or — , majority of strains positive, occasional cultures negative
- or + , majority of cultures negative, occasional strains positive
- (+) or + , majority of reactions delayed, some occur within 1 or 2 days
- d , different reactions: +, (+), —

²Numerals in parentheses indicate percentage of delayed reactions (3 or more days)

Table 17a.—Biochemical tests obtained with cultures of *Enterobacter liquefaciens*
(Commonly employed tests)

Test or substrate	Number					Percent				
	+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14		1-2	3-7	8-14	>14	
Hydrogen sulfide	0	2 ^w	66	0	2.9	97.1
Urease	3 ^w	13	52	4.4	19.1	76.5
Indol	0	68	0	100
Methyl red:										
37 C	51	17	75	25
22 C (63)	21	42	33.3	66.7
Voges-Proskauer:										
37 C	21	47	30.9	69.1
22 C (63)	50	13	79.4	20.6
Citrate (Simmons')	62	5	1	91.2	7.3	1.5
KCN	67	1	98.5	1.5
Motility:										
37 C	54	12	2	79.4	17.7	2.9
22 C	68	0	100	0
Gelatin:										
22 C	67	1	0	98.5	1.5	0
Lysine decarboxylase:										
37 C	67	12	82.4	17.6
22 C (63)	63	0	100	0
Arginine dihydrolase:										
37 C	3	65	4.4	95.6
22 C (63)	0	63	0	100
Ornithine decarboxylase:										
37 C	67	1	98.5	1.5
22 C (63)	63	0	100	0
Phenylalanine deaminase	1 ^w	67	1.5	98.5
Glucose:										
acid	68	0	100	0
gas	64	1	3	94.1	1.5	4.4
Lactose:										
37 C	1	14	5	1	47	1.5	20.6	7.3	1.5	69.1
22 C (62)	1	48	6	3	4	1.6	77.4	9.7	4.8	6.5
Sucrose:										
acid	68	0	100	0
Dulcitol	0	68	0	100
Salicin	68	0	100	0
Adonitol:										
acid	6	2	60	8.8	2.9	88.3
gas	1	1	66	1.5	1.5	97
Inositol:										
acid	66	1	1	97	1.5	1.5
gas	1	15	52	1.5	22	76.5
Sorbitol:										
acid	66	2	97	3
gas	55	1	12	80.9	1.5	17.6
Arabinose:										
37 C	63	5	92.6	7.4
22 C (56)	53	3	94.6	4.4
Raffinose:										
acid	59	2	7	86.8	2.9	10.3
37 C gas	12	39	2	15	17.6	57.4	2.9	22.1
22 C acid (55)	50	2	2	1	90.9	3.6	3.6	1.9
22 C gas (55)	50	5	90.9	9.1
Rhamnose	0	68	0	100

w = weakly positive reactions

Table 17b.—*Reactions obtained with strains of Enterobacter liquefaciens*
(Additional biochemical tests)

Test or substrate	Number					Percent				
	+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14		1-2	3-7	8-14	>14	
Malonate.....	5	63	7.4	92.6
Mucate.....	0	68	0	100
Nitrite from nitrate.....	68	0	100	0
Organic acids ¹										
citrate.....	44	24	64.7	35.3
D-tartrate.....	15	53	22.1	77.9
Jordan's tartrate (60).....	45	15	75	25
Sodium alginate:										
22 C (64).....	0	64	0	100
37 C (64).....	0	64	0	100
Lipases (57):										
corn oil 37 C.....	49	5	3	86	8.8	5.2
22 C.....	56	1	0	98.2	1.8	0
Triacetin 37 C.....	11	23	19	4	19.3	40.4	33.3	7
22 C.....	20	20	8	9	35.1	35.1	14	15.8
Tributyrin 37 C.....	47	3	7	0	82.5	5.3	12.2	0
22 C.....	52	1	4	91.2	1.8	7
Pectate (64):										
37 C.....	0	64	0	100
22 C.....	0	64	0	100
Starch.....	17	15	20	11	5	25	22.1	29.4	16.2	7.3
Xylose:										
37 C.....	63	1	4	92.6	1.5	5.9
22 C (55).....	50	5	90.9	9.1
Cellobiose:										
acid.....	18	15	15	20	26.5	22.1	22	29.4
gas.....	4	13	10	41	5.9	19.1	14.7	60.3
Glycerol:										
acid.....	67	1	0	98.5	1.5	0
gas.....	31	25	1	11	45.6	36.7	1.5	16.2
Alpha methyl glucoside (60):										
37 C.....	13	47	21.7	78.3
22 C.....	23	35	2	38.3	58.4	3.3
Erythritol (60):										
37 C.....	0	60	0	100
22 C.....	0	60	0	100
Esculin (60):										
37 C.....	44	1	15	73.3	1.7	25
22 C.....	36	16	8	60	26.7	13.3
Beta galactosidase (39).....	39	0	100	0

¹Method of Kauffmann and Petersen, 1956

Table 18.—*Patterns of reactions obtained with cultures of Enterobacter liquefaciens in certain differential tests and reactions given by strains that were somewhat aberrant*

Number of cultures in each group	Lysine	Arginine	Ornithine	Inositol (gas)	Adonitol (gas)	Glycerol (gas)	Gelatin	Motility	Number of aberrant strains	Deviations noted
33	+	-	+	-	-	+	+	+	1	1 raffinose (+)
13	+	-	+	+	-	+	+	+	4	1 arabinose -, raffinose -, adonitol +(37 C); 1 arabinose -, raffinose -, xylose (+), adonitol +(37 C); 1 xylose -, raffinose -, adonitol +(37 C); 1 arabinose -, xylose -, raffinose -, adonitol +(37 C)
6	+	-	+	-	-	-	+	+
1	+	-	+	+	-	-	+	+	1	1 adonitol +
1	+	-	+	+	+	+	+	+	1	1 adonitol +
1	+	-	+	+	+	-	+	+	1	1 arabinose -, xylose -, raffinose -, adonitol +
1	+	-	-	-	-	-	+	+	1	1 sorbitol -, adonitol A +
1	-	+	+	-	-	-	+	+	1	1 KCN -, arabinose -, xylose -, raffinose -, sorbitol -, citrate -
1	-	+	+	-	-	+	+	+
9	-	-	+	-	-	+	+	+	1	1 raffinose -
1	-	-	-	-	-	-	+	+	1	1 inositol -

Table 19.—*Additional reactions given by cultures of Enterobacter liquefaciens on certain substrates*

Substrate	37 C						22 C					
	No. tested	+	(+) 3-7	(+) 8-14	(+) >14	-	No. tested	+	(+) 3-7	(+) 8-14	(+) >14	-
Alpha methyl glucoside:												
Acid	60	13	47	60	23	35	2
Gas	2	58	1	25	34
Arabinose:												
Acid	68	63	5	56	53	3
Gas	16	10	42	51	5
Cellobiose:												
Acid	68	18	15	15	...	20	47	5	42	0
Gas	4	13	10	...	41	3	44	0
Glycerol:												
Acid	68	67	1	0	47	47	0
Gas	31	25	1	...	11	47	0
Erythritol:												
Acid	60	0	60	60	0	60
Gas	0	60	0	60
Esculin:												
Acid	60	44	1	15	60	36	16	8
Gas	22	2	36	17	22	21
Inositol:												
Acid	68	66	1	1	47	46	1	0
Gas	1	15	52	3	43	1	0
Lactose:												
Acid	68	1	14	5	1	47	62	1	48	6	3	4
Gas	0	1	16	1	52	0	1	35	10	5
Raffinose:												
Acid	68	59	2	7	55	50	2	2	...	1
Gas	12	39	15	50	5
Xylose:												
Acid	68	63	1	4	55	50	4	1
Gas	21	13	3	...	31	50	5

Note: Of 57 strains tested, none was able to liquefy nor utilize alginate at either temperature.

Table 20a. — Differentiation of species of *Enterobacter* (Commonly employed biochemical tests)

Test or substrate	<i>E. cloacae</i>		<i>E. aerogenes</i>		<i>E. alvei</i>		<i>E. liquefaciens</i> ¹	
	Index ²	%+ (%) ³	Index ²	%+ (%) ³	Index ²	%+ (%) ³	Index ²	%+ (%) ³
Hydrogen sulfide (TSI)	—	0	—	0	—	0	—	0
Urease	+or—	64.7	—	2.7	—	3	d	4.4(19.1)
Indol	—	.5	—	0	—	0	—	0
Methyl red:								
(37 C)	—	3	—	0	+or—	54	+or—	75
(22 C)	—	—	—	—	—	1	—or+	33.3
Voges-Proskauer:								
(37 C)	+	99.5	+	100	+or—	65	—or+	30.9
(22 C)	+	99.5	+	100	+	99	+or—	79.4
Citrate (Simmons'):								
(37 C)	+	99.5	+	93.7	(+)or—	(58)	+	91.2(7.3)
(22 C)	—	—	—	—	d	3(79)	—	—
KCN (growth)	+	98	+	98.7	+	96(1)	+	98.5
Motility:								
(37 C)	+	94.5	+	97.3	+	93	d	79.4(17.7)
(22 C)	—	—	—	—	—	—	+	100
Gelatin (22 C)	(+)	1(96)	(+)or—	(77.3)	—	0	+	98.5(1.5)
Lysine decarboxylase	—	.5	+	98.7	+	100	+or—	82.4
Arginine dihydrolase	+	96.5	—	0	—	9	—	4.4
Ornithine decarboxylase	+	96	+	98.7	+	100	+	98.5
Phenylalanine deaminase	—	0	—	0	—	0	—	1.5
Glucose:								
Acid	+	100	+	100	+	100	+	100
Gas	+	100	+	100	+	100	+	94.1(1.5)
Lactose	+	93.5(5.5)	+	92.1(5.3)	—or(+)	(23)	d	1.5(29.4)
Sucrose	+	96.5(.5)	+	100	d	12(61)	+	100
Mannitol	+	100	+	100	+	100	+	100
Dulcitol	—or+	12.9	—	4	—	1	—	0
Salicin	d	75.6(18.4)	+	98.7(1.3)	d	13(8)	+	100
Adonitol:								
Acid	—or+	28.4	+	98.7	—	0	d	8.8(2.9)
Gas	—or+	28.4	+	98.7	—	0	—	1.5(1.5)
Inositol:								
Acid	d	21.9(12.4)	+	100	—	0	+	97(1.5)
Gas	—	4.5	+	100	—	0	d	1.5(22)
Sorbitol	+	94.5(.5)	+	100	—	0	+	97
Arabinose	+	99.5	+	100	+	96	+	92.6
Raffinose	+	97	+	96	—	0	d	86.8(2.9)
Rhamnose	+	92(1.5)	+	98.7	+	93(7)	—	0

¹See table 19 for additional data on reactions obtained by incubation at 22 C²Index:

+, positive within one or two days' incubation

(+) , positive reaction after 3 or more days

—, no reaction

+or—, majority of strains positive, occasional cultures negative

—or+, majority of cultures negative, occasional strains positive

(+)or+, majority of reactions delayed, some occur within 1 or 2 days

d, different reactions: +, (+), —

³Numerals in parentheses indicate percentage of delayed reactions (3 or more days)

Table 20b.—Differentiation of species of *Enterobacter* (Additional biochemical tests)

Test or substrate	<i>E. cloacae</i>		<i>E. aerogenes</i>		<i>E. alvei</i>		<i>E. liquefaciens</i> ¹	
	Index ²	%+ (%+) ³	Index ²	%+ (%+) ³	Index ²	%+ (%+) ³	Index ³	%+ (%+) ³
Malonate	+ or -	80.6	+ or -	74.7	+ or -	74	-	7.4
Mucate	+ or -	75.6	+	94.7	-	0	-	0
Nitrite from nitrate	+	100	+	100	+	100	+	100
Organic acids ⁴ :								
Citrate	+ or -	86.1	+	97.3	+	99.1	+ or -	64.7
D-tartrate	- or +	16.9	- or +	40	-	0	- or +	22.1
Jordan's tartrate	- or +	27.8	+ or -	89.3	+ or -	75.4	+ or -	75
Sodium alginate, synthetic	-	0	-	0	-	0	-	0
nutrient	-	0	-	0	-	0	-	0
Lipases:								
corn oil	-	.8(1.5)	-	0	-	0	+ or (+)	86(8.8)
triacetin	-	0	-	2	-	0	d	19.3(73.7)
tributyrin	-	(.8)	-	0	-	0	+ or (+)	82.5(17.5)
Pectate	-	0	-	0	-	0	-	0
Starch	d	15.9(33.4)	d	38.7(20)	d	50(16)	d	25(67.7)
Xylose	+	98(5)	+	100	+	97	+	92.6(1.5)
Cellobiose:								
acid	+	100	+	98.7	d	76(16)	d	26.5(44.1)
gas	+	100	+	98.7	d	67(22)	d	5.9(33.8)
Glycerol:								
acid	d	43.3(44.8)	+	100	+	100	+	98.5(1.5)
gas	d	5.5(15.9)	+	98.7(1.3)	+	95(5)	d	45.6(38.2)
Alpha methyl glucoside	+ or (+)	84.2(12.8)	+	96(2)	-	0	- or +	21.7
Erythritol	-	0	-	0	-	0	-	0
Esculin	- or +	29.3	+	98	-	6(2)	d	73.3(1.7)

¹See table 19 for additional data on reactions obtained by incubation at 22 C²Index:

+, positive within 1 or 2 days' incubation

(+), positive reaction after 3 or more days

-, no reaction

+ or -, majority of strains positive, occasional cultures negative

- or +, majority of cultures negative, occasional strains positive

(+) or +, majority of reactions delayed, some occur within 1 or 2 days

d, different reactions: +, (+), -

³Numerals in parentheses indicate percentage of delayed reactions (3 or more days)⁴Method of Kauffmann and Petersen, 1956

Table 21.—*Differentiation of common species of Klebsiella and Enterobacter*¹

Test or substrate	<i>K. pneumoniae</i>		<i>E. cloacae</i>	
	Index ²	%+ (%+) ³	Index ²	%+ (%+) ³
Gas from:				
inositol	+	91.9	—	4.5
glycerol	+	92.5	—	5.5
adonitol	+ or —	83.7	— or +	28.4
Esculin	+	98.9	— or +	29.3
	(1.1)
Lysine decarboxylase	+	97.2	—	.5
Arginine dihydrolase	—	.9	+	96.5
Ornithine decarboxylase	—	0	+	96
Urease	+	94.5	+ or —	64.7
Gelatin 22 C	—	3.3	(+)	96
Motility	—	0	+	94.6
Growth on synthetic alginate medium	+ or (+)	88.5	—	0
	(9.2)

¹Common among materials submitted for identification (see text)²Index:

+, positive reaction within 1 or 2 days

(+), delayed reaction, 3 or more days

+ or —, majority of strains positive, occasional cultures negative

— or +, majority of cultures negative, occasional strains positive

³Numerals in parentheses indicate percentage of delayed reactions (3 or more days)

Table 22a. — Biochemical reactions obtained with cultures of *Serratia marcescens subspecies marcescens* (Commonly used tests)

Substrate or test	Number Cultures tested	Number					Percent				
		+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
		1-2	3-7	8-14	>14		1-2	3-7	8-14	>14	
Hydrogen sulfide (TSI)	323	0	78 ^w	85 ^w	...	160	0	24.2	26.3	..	49.5
Urease	435	56	119	42	...	218	12.9	27.4	9.6	..	50.1
Indol	456	0	456	0	100
Methyl red:											
(37 C)	456	61	395	13.4	86.6
(22 C)	395	34	361	8.6	91.4
Voges-Proskauer:											
(37 C)	456	456	0	100	0
(22 C)	398	398	0	100	0
Simmons' citrate	456	450	3	3	98.7	.77
KCN	328	325	3	99.19
Motility	456	448	8	98.3	1.7
Gelatin (22 C)	455	443	12	0	97.4	2.6	0
Lysine decarboxylase	456	454	2	99.64
Arginine dihydrolase	456	13 ^w	443	2.8	97.2
Ornithine decarboxylase	456	454	2	99.64
Phenylalanine deaminase	330	9 ^w	321	2.7	97.3
Glucose acid	456	456	0	100	0
Gas from glucose ¹	456	277	179	60.8	39.2
Lactose	343	9	48	...	286	2.6	14	..	83.4
Sucrose	374	373	1	99.73
Mannitol	352	352	0	100	0
Dulcitol	450	0	450	0	100
Salicin	348	331	6	11	95.1	1.7	3.2
Adonitol	438	207	98	133	47.2	22.4	30.4
Inositol	438	321	57	60	73.3	13	13.7
Sorbitol	434	427	7	98.4	1.6
Arabinose	453	1	452	.2	99.8
Raffinose	451	4	11	436	.9	2.4	96.7
Rhamnose	452	0	452	0	100

¹ When gas was formed, the volumes were small (about 10% or less).

^w= weakly positive reaction

Table 22b. — Reactions obtained with strains of *Serratia marcescens subspecies marcescens*
(Additional biochemical tests)

Substrate or test	Number Cultures tested	Number					Percent				
		+	(+)	(+)	(+)	—	+	(+)	(+)	(+)	—
		1-2	3-7	8-14	>14		1-2	3-7	8-14	>14	
Malonate	289	5	284	1.7	98.3
Mucate	269	0	269	0	100
Christensen's citrate	249	244	3	2	98	1.28
Organic acids ¹ :											
citrate	47	47	0	100	0
D-tartrate	47	1 ^w	...	9 ^w	...	37	2.1	...	19.1	..	78.7
Jordan's tartrate	186	185	1	99.55
Sodium acetate	49	44	5	89.8	10.2
Ammonium salts glucose agar	109	108	1	99.19
Sodium alginate, synthetic	457	0	...	17	...	440	0	...	3.7	..	96.3
nutrient	457	0	457	0	100
Lipases:											
corn oil	446	437	4	5	98	.9	1.1
Triacetin	14	59	373	3.2	13.2	83.6
Tributyrin	400	36	10	89.7	8.1	2.2
Maltose	311	302	4	5	97.1	1.3	1.6
Xylose	339	27	44	18	...	250	8	13	5.3	..	73.7
Trehalose	307	306	1	0	99.7	.3	0
Cellobiose ²	428	131	137	35	...	125	30.6	32	8.2	..	29.2
Glycerol ²	414	393	18	3	94.9	4.47
Alpha-methyl glucoside	49	0	49	0	100
Erythritol	49	0	1	48	0	2	98
Esculin	49	44	5	90.8	10.2
Beta-d-galactosidase	44	44	0	100	0
Phenylpropionic acid agar	160	3 ^w	157	1.9	98.1
Cetrimide agar	210	30	7	173	14.3	3.3	82.4
Nitrate reduction	331	317	14	95.8	4.2
Oxidase	242	0	242	0	100
Pigment	456	123	333	27	73

¹ Method of Kauffmann and Petersen, 1956

² Gas not formed

^w = weakly positive reaction

Table 23.—*Patterns of reactions given by cultures of Serratia marcescens subspecies marcescens in certain differential tests and reactions given by strains that were somewhat aberrant*

Number of cultures in each group	Lactose	Arabinose	Raffinose	Voges-Proskauer	Malonate	Gelatin	Lysine	Arginine	Ornithine	gas +	Deviations noted
319	-	-	-	+	-	+	+	-	+	193	2 maltose (+), 5 maltose -, 2 raffinose +, 9 raffinose (+), 5 sorbitol -, 36 salicin + weak, 4 salicin (+), 10 salicin -, 1 trehalose (+), 3 glycerol + weak, 16 glycerol (+), 3 glycerol -, 6 Simmons' citrate + weak; 3 Simmons' citrate (+), 3 Simmons' citrate -, 1 Christensen's citrate (+), 14 nitrite -, 10 gelatin (+), 7 phenylpyruvic acid + weak, 1 KCN -
39	-	-	-	+	-	+	+w	-	+	21	3 salicin + weak, 1 glycerol -, 3 ornithine decarboxylase + weak
48	(+)	-	-	+	-	+	+	-	+	35	2 maltose (+), 1 raffinose +, 2 raffinose (+), 1 sorbitol -, 2 glycerol (+), 2 KCN -
8	-	(+)	-	+	-	-	+	-	+	6	1 lactose (+), 1 sucrose -, 1 raffinose (+)
3	-	-	-	+	+	+	+	-	+	2	1 phenylpyruvic acid + weak
2	(+)	-	-	+	+	+	+	-	+	1
8	-	-	-	+	-	+	+	(+)	+	5	2 glycerol + weak
1	+	+	+	+	-	+	+ ²	+ ²	+ ³	...	1 sorbitol -
1	-	-	-	+	-	+	-	-	-

Table 24a. — *Reactions given by cultures of Serratia marcescens subspecies kiliensis*
(Commonly used biochemical tests)

Test or substrate	Number				
	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	>14	
Hydrogen sulfide	0	4 ^w	10
Urease	2	2 ^w	10
Indol	0	14
Methyl red (37 C) or (22 C)	14	0
Voges-Proskauer (37 C) or (22 C)	0	14
Citrate (Simmons')	10	3	1	...	0
KCN	13	1
Motility	11	3
Gelatin (22 C)	12	2	0
Lysine decarboxylase	12	2
Arginine dihydrolase	0	14
Ornithine decarboxylase	14	0
Phenylalanine deaminase	0	14
Glucose acid	14	0
Gas from glucose	1	13
Lactose	0	3	2	1	8
Sucrose	13	1	0
Mannitol	14	0
Dulcitol	0	14
Salicin	14	0
Adonitol	5	2	7
Inositol	8	4	2
Sorbitol	14	0
Arabinose	0	14
Raffinose	0	14
Rhamnose	0	14

^w=weakly positive reaction

Table 24b. — *Biochemical reactions given by strains of Serratia marcescens subspecies kiliensis*
(Additional biochemical tests)

Test or substrate	Number				
	+	(+)	(+)	(+)	—
	1-2	3-7	8-14	<14	
Malonate	0	14
Mucate	0	14
Christensen's citrate	14	0
Organic acids ¹					
citrate	14	0
D-tartrate	2 ^w	12
Jordan's tartrate	13	1
Sodium acetate	5	6	3
Ammonium salts glucose agar	9	4	1	...	0
Sodium alginate	0	14
Lipases:					
corn oil	14	0
Triacetin	1	13
Tributyrin	14	0
Maltose	14	0
Xylose	1	2	1	...	10
Trehalose	14	0
Cellobiose	9	2	2	...	1
Glycerol	12	2	0
Alpha methyl glucoside	0	14
Erythritol	0	1	7	...	6
Esculin	13	1
Beta galactosidase	14	0
Phenyl propionic acid agar	0	14
Cetrimide agar	3	1	10
Nitrite from nitrate	14	0
Oxidase	0	14
Pigment	3	11

¹ Method of Kauffmann and Petersen, 1956

^w = weakly positive reaction

Table 25 — Differentiation of *Serratia marcescens* and *Enterobacter liquefaciens*

Substrate or test	<i>E. liquefaciens</i>			<i>S. marcescens</i>		
	Index ¹	%+	(%+) ²	Index ¹	%+	(%+)
Glucose:						
Acid	+	100	+	100
Gas	+	94.1	+ ³ or -	61.3
Inositol:						
Acid	+	97	(1.5)	d	73.8	(12.6)
Gas	d	1.5	(22)	-	0	0
Glycerol:						
Acid	+	98.5	(1.5)	+	94.6	(4.4)
Gas	d	45.6	(38.2)	-	0	0
Cellobiose:						
Acid	d	26.5	(44.1)	d	30.6	(40.2)
Gas	d	5.9	(33.8)	-	0	0
Esculin:						
Acid	d	75	(1.6)	d	71.3	(.2)
Gas	-or+ ⁴	37.5	-	0	0
Raffinose:						
Acid	d	86.8	(2.9)	-	.9	(2.6)
Gas	d	17.6	(60.3)	-	0	0
Arabinose:						
Acid	+	92.6	-	.2	(1.9)
Gas	d	23.5	(14.7)	-	0	0
Xylose:						
Acid	+	92.6	(1.5)	d	7.9	(18.6)
Gas	d	30.9	(23.5)	-	0	0
Erythritol:						
Acid	-	0	d	1.7	(22.8)
Alpha methyl glucoside:						
Acid	-or+	21.7	-	.9	(.6)
Methyl red:						
(37 C)	+or-	75	-or+	14
(22 C)	-or+	33.3	-or+	8.8
Voges-Proskauer:						
(37 C)	-or+	30.9	+	100
(22 C)	+or-	79.4	+	100

¹Index:

- +, positive within 1 or 2 days' incubation
 (+), positive reaction after 3 or more days
 -, no reaction
 +or-, majority of strains positive, occasional cultures negative
 -or+, majority of cultures negative, occasional strains positive
 (+)or-, majority of reactions delayed, some occur within 1 or 2 days
 d, different reactions: +, (+), -

²Numerals in parentheses indicate percentage of delayed reactions (3 or more days)³Gas volumes 10 percent or less⁴Gas volumes: bubble to 10 percent

Table 26.—Differentiation of species of *Klebsiella*, *Enterobacter*, and *Serratia*

Test or substrate	<i>Klebsiella pneumonia</i>		<i>Enterobacter</i>								<i>Serratia marcescens</i> subsp. <i>marcescens</i>	
			<i>cloacae</i>		<i>aerogenes</i>		<i>alvei</i>		<i>liquefaciens</i>			
	Index ¹	%+	Index ¹	%+	Index ¹	%+	Index ¹	%+	Index ¹	%+	Index ¹	%+
Gas from:												
glucose	+	96.5	+	100	+	100	+	100	+	94.1	+ ² or -	61.3
adonitol	+ or -	83.7	- or +	28.4	+	98.7	-	0	-	1.5	-	0
inositol	+	91.9	-	4.5	+	100	-	0	d	23.5	-	0
glycerol	+	92.5	-	5.5	+	100	+	100	d	82.3	-	0
cellobiose	+	95.7	+	100	+	98.7	d	88	d	25	-	0
Sorbitol	+	99.4	+	94.5	+	100	-	0	+	97	+	98.3
Raffinose	+	99.7	+	97	+	96	-	0	d	89.7	-	.9
Rhamnose	+	99.3	+	92	+	98.7	+	93	-	0	-	0
Arabinose	+	99.9	+	99.5	+	100	+	96	+	92.6	-	.2
Methyl red:												
37 C	- or +	13.3	-	.3	-	0	+ or -	54	+ or -	75	- or +	14
22 C							-	1	- or +	25	-	8.8
Voges-Proskauer:												
37 C	+	91.1	+	99.5	+	100	+ or -	65	- or +	30.9	+	100
22 C							+	99	+ or -	79.4	+	100
Lysine												
decarboxylase	+	97.2	-	.5	+	98.7	+	100	+ or -	82.4	+	99.8
Arginine												
dihydrolase	-	.9	+	96.5	-	0	-	9	-	4.4	-	3
Ornithine												
decarboxylase	-	0	+	96	+	98.7	+	100	+	98.5	+	99.8
Malonate	+	92.5	+ or -	80.6	+ or -	74.7	+ or -	74	-	7.4	-	1.9
Mucate	+	92.8	+ or -	75.6	+	94.7	-	0	-	0	-	0
Urease	+	94.5	+ or -	64.7	-	2.7	-	3	d	23.5	d	52.4
Gelatin 22 C	-	3.3	(+)	(96)	(+) or -	(77.3)	-	0	+	100	+	97.2
Motility	-	0	+	94.5	+	97.3	+	93	+	97.1	+	99.1
Growth on synthetic . .	+ or (+)	88.5	-	0	-	0	-	0	- or (+)	(20.3)	-	0
alginate medium		(9.2)										

¹Index:

+, positive reaction within 1 or 2 days

(±), delayed reaction, 3 or more days

d, different biochemical reactions. In this table (only) the percentages given with the symbol "d" are based upon the positive reactions obtained within 7 days of incubation

²When gas is formed from glucose by *Serratia*, the volumes are small (10% or less)

Table 27.—Additional reactions of members of the tribe KLEBSIELLEAE on certain substrates¹

Species	No. tested	Alpha methyl glucoside			Erythritol			Esculin		
		+	(+) 3-7	-	+	(+) 3-7	-	+	(+) 3-7	-
<i>K. pneumoniae</i> :										
acid.....	95	82(86)	13(14)	0	0	95	94(99)	1(1)	0
gas.....	95	82(86)	11(12)	2(2)	92(97)	3(3)
<i>K. ozaenae</i> :										
acid.....	53	33	13	7	0	53	42	3	8
gas.....	53	17	10	27	0	53	22	4	27
<i>K. rhinoschleromatis</i> :										
acid.....	22	0	22	0	22	21	1	0
gas.....	22	0	22	0	22	0	22
<i>E. cloacae</i> :										
acid.....	133	112(84)	17(13)	4(3)	0	133	39(29)	94(71)
gas.....	133	106(80)	13(10)	14(10)	37(28)	96(72)
<i>E. aerogenes</i> :										
acid.....	49	47	1	1	0	49	48	1	0
gas.....	49	46	2	1	48	1	0
<i>E. alvei</i> :										
acid 37 C.....	100	0	100	0	100	6	2	92
gas 37 C.....	100	5	2	93
acid 22 C.....	100	0	100	0	100	2	10	88
gas 22 C.....	100	2	10	88
<i>Serratia marcescens</i> :										
acid.....	464	4(.9)	3(.6)	457(98.5)	8(1.7)	106(22.8)	350(75.5)	331(71.3)	1(.2)	132(28.5)
gas.....	464	0	464	0	464	0	464

¹See table 19 for reactions given by *E. liquefaciens*.

N.B., numerals in parentheses are percentages of numbers tested

Table 28.—Biochemical tests of value in the differentiation of species and subspecies of Klebsiellae that give or may give negative Voges-Proskauer reactions

Test or substrate	<i>K. ozaenae</i>		<i>K. rhinoschleromatis</i>	<i>E. alvei</i>		<i>E. liquefaciens</i>		<i>S. marcescens</i> , subsp. <i>kiliensis</i>
	Index ¹	%+	Index ¹	Index ¹	%+	Index ¹	%+	Index ¹
Methyl red:								
37C.....	+	99	+	+ or -	54	+ or -	75	+
22C.....	-	1	- or +	33	+
Voges-Proskauer:								
37C.....	-	0	-	+ or -	65	- or +	31	-
22C.....	+	99	+ or -	79	-
Citrate (Simmons ¹): 37C.....	d	31(31)	-	(+) or -	(58) ^a	+	91(7)	d
Motility.....	-	0	-	+	93	d	79(18) ^b	d
Gelatin: 22C.....	-	0	-	-	0	+	98(2)	+ or (+)
Lysine decarboxylase:								
37C.....	- or +	49	-	+	100	+ or -	82	+ or -
22C.....	+	100	+	100
Arginine dihydrolase:								
37C.....	-	6	-	-	9	-	4	-
22C.....	-	5	-	0

(See footnotes at end of table)

Table 28.—Biochemical tests of value in the differentiation of species and subspecies of Klebsiellae that give or may give negative Voges-Proskauer reactions—Continued

Test or substrate	<i>K. ozaenae</i>		<i>K. rhinoschleromatis</i>	<i>E. alvei</i>		<i>E. liquefaciens</i>		<i>S. marcescens</i> , subsp. <i>kiliensis</i>
	Index ¹	%+	Index ¹	Index ¹	%+	Index ¹	%+	Index ¹
Ornithine decarboxylase:								
37C	—	2	—	+	100	+	98	+
22C	+	100	+	100
Gas from glucose	d	68(3)	—	+	100	+	94(2)	— or +
Lactose	d	24(71)	(+) or —	— or (+)	(23)	d	2(29) ^b	(+) or —
Sucrose	d	16(18)	+ or (+)	d	73	+	100	+ or (+)
Adonitol:								
acid	+	99(1)	+	—	0	d	9(3)	d
gas	d	59(2)	—	—	0	—	2(1)
Inositol:								
acid	d	60(21)	+ or (+)	—	0	+	97(2)	d
gas	d	28(8)	—	—	0	d	2(22)	—
Cellobiose:								
acid	+	98	+	d	76(16)	d	27(44)	d
gas	+ or —	71	—	d	67(22)	d	6(34)	—
Glycerol:								
acid	d	69(21)	+	+	100	+	98(2)	+ or (+)
gas	d	31(24)	—	+	95(5)	d	46(38)	—
Sorbitol	d	78(10)	+	—	0	+	97	+
Arabinose	+	100	+	+	96	+	95	—
Raffinose	d	88(2)	+ or (+)	—	0	d	87(3) ^b	—
Rhamnose	d	63(8)	+ or (+)	+	93(7)	—	0	—
Alpha methyl glucoside	d	62(25)	—	—	0	— or +	22 ^b	—
Esculin:								
acid	d	79(6)	+ or (+)	—	6(2)	d	75(2)	+ or —
gas	d	42(8)	—	—	5(2)	d	75(2)	—
Malonate	—	6	+ or —	+ or —	74	—	7	—
Mucate	— or +	26	—	—	0	—	0	—
Organic acids ² :								
citrate	— or +	18	—	+	99	+ or —	64.7	+
D-tartrate	— or +	39	—	—	0	— or +	22	— or + ^w
Lipases:								
corn oil:								
37C	—	0	—	—	0	d	86(9)	+
22C	—	0	—	0	+	98(2)
Triacetin	—	0	—	—	0	d	19 ^b	— or +
Pigment	—	0	—	—	0	—	0	— or +

N.B. Numerals indicate percentages of positive reactions that occurred within 1 or 2 days. Numerals in parentheses are percentages of delayed positive (3 or more days) reactions.

¹Index:

- +, positive reaction within 1 or 2 days
- (+), delayed positive (3 or more days)
- , negative test
- + or —, majority of cultures positive, some strains negative
- or +, majority of strains negative, some cultures positive
- d, different biochemical reactions
- w, weakly positive

²Method of Kauffmann and Petersen, 1956.

^aWhen incubated at 22 C, 82% of *E. alvei* (Hafnia) cultures gave positive reactions on Simmons' citrate medium.

^bWhen incubated at 22 C, 100% of *E. liquefaciens* strains were motile, 91.9% fermented lactose (88.7% in 1 to 14 days), 90.9% fermented raffinose, 35% hydrolysed triacetin, and 38% fermented alpha methyl glucoside rapidly and 58% produced acid from it in 3 or more days of incubation.

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